Leveraging Recombinant Vault Nanoparticles Produced in *Komagataella phaffii* for Targeted Delivery of siRNAs

Giulia Tomaino[1-2], Camilla Pantaleoni[1], Annalisa D'Urzo[1], Annie Dutriaux[2], Domenico Flagiello[2], Marco Vanoni[1-3], Paolo Tortora[1], Gianni Frascotti[1]

E-mail: g.tomaino3@campus.unimib.it

[1] Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milano, Italy

[2] Université Paris Cité, CNRS, Institut Jacques Monod, F-75013 Paris, France

[3] ISBE-SYSBIO Centre of Systems Biology, Milan, Italy.

Keywords: Vault nanoparticle, *K. phaffii*, targeted delivery, siRNA delivery, cancer treatment

Abstract:

Recently, nanotechnology has significantly impacted medicine, particularly through the therapeutic and diagnostic potential of nanoparticles (NPs). Protein-based NPs have gained attention as drug delivery systems and vaults emerge as ideal candidates due to their have unique qualities such as non-immunogenicity, biodegradability, dynamic structure, and a spacious cavity. Vaults are the largest known ribonucleoprotein particles naturally occurring in higher eukaryotic cells. 78 copies of the dominant component Major Vault Protein (MVP) assemble into a barrel-like "nano-capsule", enclosing other proteins like poly (ADP-ribose) polymerase, telomerase-associated protein-1, and some small untranslated RNAs. Vaults are associated with cellular functions promoting cell survival and providing cytoprotective effects. Current production and purification methods for vaults are complex, partly due to their reliance on higher eukaryotes for expression. We here present a simplified procedure that involves expressing human vaults in the yeast *Komagataella phaffii* and using a streamlined purification process including RNase pretreatment and size-exclusion chromatography.

To achieve specific targeting, NPs can be conjugated to antibodies (Ab) on the surface by different chemical methods, that not necessarily guarantee the correct orientation of the Ab to be active. Here we address this challenge with the construction of an engineered vault variant carrying the protein A-derived Z peptide - tightly binding Fc portion of human IgG1 - allowing for direct and oriented Ab-vault conjugation. Vault-Z maintains the same morphology and size as the wild-type vault. A comprehensive characterization of vault-Z:Ab binding reveals the capacity to bind up to 10-12 Ab molecules per vault-Z. Lastly, we shown that vault-Z uptake in cancer cells, significantly increases upon conjugation with a targeting Ab.

The rise of nucleic acid-based therapeutics has demanded better delivery systems. We here investigate the potential of using vaults for siRNA delivery, focusing on siRNAs targeting *LADON*, a long-non-coding RNA associated with tumor progression in melanoma. Aiming for the direct loading of small-non-coding RNA (siRNA) into vaults we demonstrate that recombinant vaults can mediate the delivery of naked siRNA molecules, without additional formulation aiding the transfection, causing a biological effect.